



Gastric GISTs: Analysis of *c-Kit*, *PDGFRA* and *BRAF* mutations in relation to prognosis and clinical pathological characteristics of patients — A GIRCG study

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Abstract

Background: Gastric gastrointestinal stromal tumors (GISTs) represent a subgroup of GISTs with a better prognosis than those located in other areas. In this retrospective study we performed a molecular characterization of a large series of patients with gastric GISTs in relation to clinical–pathological characteristics and prognosis.

Methods: DNA was extracted from paraffin-embedded sections from 221 gastric GIST patients submitted to surgery. Exons 9, 11, 13 and 17 of *KIT*, exons 12 and 18 of *PDGFRA* and exons 11 and 15 of *BRAF* were analyzed by direct sequencing. Cox regression analysis adjusted for clinical–pathological factors was performed to evaluate *KIT* and *PDGFRA* mutations in relation to the composite endpoint of relapse or death.

Results: *KIT* and *PDGFRA* mutations were observed in 119 (53.8%) and 56 (25.3%) patients, respectively, whereas 46 (20.8%) patients had wild type (wt) disease. Univariable analyses showed that a high Miettinen risk category and the presence of ulceration and *KIT* deletions

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were associated with increased risk of relapse or death ($p < 0.001$; $p = 0.0389$ and $p = 0.002$, respectively). After adjusting for Miettinen risk score, *KIT* deletions remained an independent prognostic factor ($HR_{adj} = 2.65$, 95% CI [1.15–6.13], $p = 0.023$). Moreover, *KIT* deletions in exon 11 codons 557, 558 or 559 were associated with a higher risk of relapse or death than wt tumors ($HR_{adj} = 3.29$ 95% CI [1.64–6.64], $p = 0.001$).

Conclusions: *KIT* deletions in exon 11, especially those involving codons 557, 558 or 559, were correlated with a more aggressive gastric GIST phenotype and increased risk of relapse or death.

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Keywords: GISTs; *KIT*; *PDGFRA*; *BRAF*; Prognostic factors; Mutation

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract and can develop in any part of this area; 60–70% of clinically manifest tumors arise in the stomach and 20–30% in the small intestine, but a small percentage also occurs in the rectum, colon, esophagus or omentum.^{1–3} About 70–80% of GISTs harbor a *KIT* gene mutation in exons 11 (about 60% of cases) and 9 (7–10%) and, less frequently, in exons 13 and 17.^{4–6} About 20–30% of *KIT* wild type (wt) GISTs show mutations in the platelet-derived growth factor receptor alpha (*PDGFRA*) gene, in particular in exons 12, 14 and 18,^{6,7} while a lower percentage (4–13%) have *BRAF* mutations.^{8–10}

Gastric GISTs represent a subgroup of GISTs with a favorable prognosis and are characterized by a relatively higher fraction of cases with an epithelioid or mixed epithelioid/spindle morphology, a higher frequency of *PDGFRA* mutations and a lower frequency of *KIT* alterations, a lower mitotic index and overall lower mortality than other GISTs.^{11–14} Whilst the predictive role of *KIT* and *PDGFRA* mutations in relation to response to imatinib is well known,^{15–17} the prognostic significance of these mutations and of the type of mutation has yet to be defined. Some studies have shown that gastric GISTs with exon 11 deletions have a worse outcome than those with single nucleotide substitutions at the same exon.^{18–20} *PDGFRA* exon 18 mutations have also been associated with a lower risk of metastasis and a better prognosis.^{12,18} Other studies have demonstrated that, in addition to different exons, the type of mutation and several codons affected by mutations may have different prognostic implications. In particular, *KIT* exon 11 deletions are associated with a risk of metastasis, while those involving codons 557–558 indicate a higher risk of progression.^{21–25} Conversely, single *KIT* exon 11 substitutions have been correlated with longer relapse-free and overall survival.^{15,21,23,25,26} Moreover, *KIT* exon 9 duplications, which occur mainly in intestinal tumors, have been associated with aggressive behavior.^{21,25}

A number of studies have also analyzed *BRAF* gene alterations in GISTs, reporting a mutation frequency of about 4–13%.^{8–10} A predominant small intestinal location of GISTs with *BRAF* V600E has been observed, followed

by a location in the stomach.⁸ *BRAF* mutations are not *per se* indicative of malignancy in that they have not been found to show a significant correlation with prognosis.⁹ However, a recent study in which GIST patients were divided into 3 prognostic groups on the basis of type of mutation found that *BRAF* mutations were associated with the group with the best prognosis, suggesting a positive prognostic effect of this alteration.²⁷

The main aim of our retrospective study was to assess *KIT*, *PDGFRA* and *BRAF* mutations in a large series of patients with gastric GISTs recruited by member centers of the Italian Research Group of Gastric Cancer (GIRCG). We analyzed different gene mutations and types of mutation in relation to the clinical–pathological characteristics of patients to see whether this information could be used to improve the clinical management of the disease.

Materials and methods

Case series

We retrospectively analyzed a cohort of 221 patients with gastric GISTs submitted to surgical resection between March 1985 and December 2012. All cases were recruited from 8 member centers of the Italian Gastric Cancer Research Group (GIRCG). Information on clinical–pathological data such as tumor size, mitosis, presence of ulceration, necrosis, atypia and type of cellularity was collected by reviewing all available medical and histopathological records archived in GIRCG centers. The study was approved by the Local Ethics Committee of each center.

Histopathological variables analyzed for each tumor were as follows: size, mitotic count per 50 high-power fields (HPF), cell type, presence or absence of ulceration, necrosis and nuclear atypia, and pattern of *KIT* and *MIB1* immunostaining. Immunohistochemical staining was performed using the following primary antibodies: *KIT* (CD117 antigen, Dako Corporation, Carpinteria, CA, USA) and Ki67 (MIB1, Dako Corporation).

On the basis of the Miettinen risk score, GISTs were stratified as no-, very low-, low-, intermediate- and high-risk tumors.²

Molecular analysis

Formalin-fixed-paraffin-embedded (FFPE) tumor samples were used for molecular analysis. For each sample, areas containing at least 50% of tumor cells were selected in hematoxylin-eosin-stained sections, macrodissected in 5- μ M sections and collected in specific tubes for DNA extraction. Tumor cells were lysed overnight at 56 °C in 50 mM of KCl, 10 mM of Tris–HCl pH 8.0, 2.5 mM of MgCl₂ and Tween-20 (0.45%) in the presence of 1.25 mg/ml of proteinase K. Proteinase K was inactivated at 95 °C for 10 min and samples were centrifuged twice at 6000 rpm to eliminate debris. DNA quantity and quality were assessed by Nanodrop (Celbio, Milan, Italy). Fifty nanograms of DNA were used for PCR amplification.

KIT exons 9, 11, 13 and 17 and *PDGFRA* exons 12 and 18 were analyzed by direct sequencing using 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Primer sequences and localizations are reported in [Table S1](#) and [Fig. S1](#), respectively.

BRAF exons 11 and 15 were analyzed by pyrosequencing using an anti-EGFR MoAb response (*BRAF*-status) kit (Diatech, Jesi, Ancona, Italy), according to the manufacturer's instructions. Reactions were run on a PyroMark Q96 ID (Qiagen, Milan, Italy).

Statistical analysis

The primary endpoint of the study was recurrence-free survival (RFS), defined as the time from the date of surgery to the date of tumor recurrence or death resulting from any cause. In patients who did not relapse or died, the end date was the last follow up. Descriptive statistics were reported as frequencies and percentages for categorical variables and as mean \pm standard deviation (SD) for continuous variables. The association between types of mutation and demographic (age and gender) and clinical–pathological features was assessed by the t-test and Chi-square test or Fisher's Exact test, as appropriate. Survival curves were estimated using the Kaplan–Meier method, while the statistical significance of the differences between curves was assessed using the log-rank test. The prognostic impact of specific variables on survival was analyzed by univariable and multivariable Cox proportional hazards models. Results are reported as Hazard Ratios (HRs) and 95% confidence intervals (95% CI). The proportional hazards assumption was checked by the Schoenfeld residuals-based test. For some variables, levels were combined due to the low number of observations.

The choice of variables to include in the multiple Cox regression model was based on stepwise selection methods and careful clinical judgment. The Akaike Information Criterion was used to select the final multiple regression models. The two multivariable Cox regression models included the Miettinen risk score as adjustment factor and the different types of mutation or the exon 11 *KIT*

mutations. Statistical interactions between demographic, clinical–pathological variables and type of mutation were explored. The prognostic role of the type of mutation was also assessed within treatment groups.

All statistical analyses were carried out using STATA 10.1 statistical software (StataCorp, College Station, TX, USA). A two-sided p-value < 0.05 was deemed statistically significant for all the analyses.

Results

Patient characteristics

Information available on the clinical–pathological characteristics of the 221 gastric GIST patients is reported in [Table 1](#). Mean age at the time of surgery was 65 \pm 13 years. One hundred and twenty patients (54.3%) were male and 101 (45.7%) were female. One hundred and sixty-two (85.7%) did not receive any type of treatment, 27 (14.3%) underwent neoadjuvant or adjuvant therapy and no information about treatment was available for 32 patients. Treated patients were similar to non treated patients as regards demographic variables but had a more serious clinical and histological profile ([Table S2](#)). In particular, they showed significantly higher tumor dimensions ($p = 0.001$) and number of mitoses ($p < 0.001$). Consequently, the Miettinen risk score was substantially higher in treated patients and necrosis and atypia were more frequent, albeit not significantly, than in non treated cases ($p = 0.091$ and $p = 0.074$, respectively).

Genotype analysis

Overall, 119 (53.8%) patients showed *KIT* mutations and 56 (25.3%) had *PDGFRA* mutations. Forty-six (20.8%) patients had *KIT* and *PDGFRA* wt GISTs. No *BRAF* mutations were detected. One hundred and sixteen of the 119 *KIT*-mutated patients showed alterations in exon 11: 47 point mutations, 43 deletions and 26 insertions. Three patients had mutations in exon 9, all of which were insertions. Five of the 56 *PDGFRA*-mutated patients had exon 12 alterations (4 point mutations and 1 deletion), while 51 showed exon 18 mutations (44 point mutations and 7 deletions).

Association between mutations and clinical–pathological characteristics of patients

The associations between the different types of mutations and pathologic tumor features are presented in [Table S3](#). The majority of *KIT* mutations displayed spindle morphology, while *PDGFRA* alterations had a mainly epithelioid phenotype (76.3% and 41.9%, respectively; $p < 0.001$). In general, the majority of GISTs carrying *KIT* or *PDGFRA* mutations had low mitotic activity ($\leq 5/50$ HPF) (85.1% and 75.2% respectively; $p = 0.052$).

Table 1
Clinical pathological characteristics of gastric GIST patients ($n = 221$).

	<i>n</i> (%)
Gender	
Male	120 (54.3)
Female	101 (45.7)
Missing	—
Age at diagnosis, years	
Mean \pm SD	65.4 \pm 13.1
Range	20–91
Missing	5
Tumor dimension, cm	
≤ 2	43 (20.3)
3–5	74 (34.9)
6–10	64 (30.2)
> 10	31 (14.6)
Missing	9
Mitoses	
≤ 5	143 (74.5)
> 5	49 (25.5)
Missing	29
Ulceration	
No	130 (83.9)
Yes	25 (16.1)
Missing	66
Necrosis	
No	118 (74.2)
Yes	41 (25.8)
Missing	62
Atypia	
No	126 (79.8)
Yes	32 (20.2)
Missing	63
Miettinen risk score	
No risk	39 (18.6)
Very low	48 (22.9)
Low	49 (23.3)
Moderate	29 (13.8)
High	45 (21.4)
Missing	11
Cellularity	
Mixed	36 (21.2)
Spindle	99 (58.2)
Epithelioid	35 (20.6)
Missing	51
Treatment	
No	162 (85.7)
Adjuvant/neoadjuvant	27 (14.3)
Missing	32

SD — standard deviation.

Overall, *KIT*-mutated patients showed worse pathological features, *i.e.*, a higher percentage of spindle cellularity ($p < 0.001$), necrosis ($p = 0.045$), and a higher Miettinen risk score ($p = 0.004$), (Table S3).

Survival analyses

The relationship between mutations and patient prognosis in terms of RFS (considered as a composite endpoint including recurrence or death, whichever came first) was evaluated in 190 patients for whom information on date

of surgery and follow up was available. Of these, 151 (79.5%) did not receive any type of treatment after surgery, while 27 (14.2%) received adjuvant or neoadjuvant therapy. Data were missing for 12 (6.3%) patients. The median follow up of patients who remained relapse-free after the last update was 72 months. There were 23 recurrences and 34 deaths from all causes without previous relapse, of which 19 from other tumors, 10 from non-cancer-related disease, one from old age and 4 from unknown causes. *KIT* mutations were detected in 100 (52.6%) of the 190 patients, while *PDGFRA* alterations were observed in 52 (27.4%) cases. Thirty-eight (20%) patients harbored the wt form of either gene. Three (3%) *KIT* mutations were exon 9 insertions, 40 (40%) were exon 11 deletions, 36 (36%) were exon 11 point mutations, and 21 (21%) were exon 11 insertions. Mutations involving codons 557, 558 or 559 were observed in 49 (49%) *KIT*-mutated patients. In particular, codons 557, 558 or 559 were involved in 22/36 (61%), 26/40 (65%) and 1/21 (5%) exon 11 point mutations, deletions or insertions, respectively (Table 2).

Among *PDGFRA* mutations, 5 (10%) were at codon 12 (4 point mutations and 1 deletion) and 47 (90%) were at codon 18 (40 point mutations and 7 deletions) (Table 2).

The association between mutation types and RFS is reported in Fig. 1.

A significantly lower RFS was observed in patients with *KIT* deletions than in those with *KIT* and *PDGFRA* wt, *PDGFRA*-mutated, and *KIT* point or insertion mutations ($p = 0.002$) (Fig. 1A). Moreover, mutations in codons 557, 558 or 559, in particular deletions, were associated with a significantly lower RFS than wt genes or mutations in other codons ($p < 0.001$) (Fig. 1B–C).

Cox univariable analyses showed that tumor dimension, number of mitoses, presence of ulceration, necrosis and a high Miettinen risk score were associated with a higher

Table 2
KIT and *PDGFRA* mutation distribution ($n = 190$).

Type of mutation	<i>n</i> (%)
wt <i>KIT</i>/<i>PDGFRA</i>	38 (20.0)
<i>KIT</i>	100 (52.6)
Exon 9:	3
Insertions	3 (1.6)
Exon 11:	97
Point mutations	36 (18.9)
Codons 557, 558 or 559	22
Deletions	40 (21.1)
Codons 557, 558 or 559	26
Insertions	21 (11.1)
Codon 557, 558 or 559	1
<i>PDGFRA</i>	52 (27.4)
Exon 12	5
Point mutations	4 (2.1)
Deletions	1 (0.5)
Exon 18	47
Point	40 (21.1)
Deletions	7 (3.7)

wt — wild type.

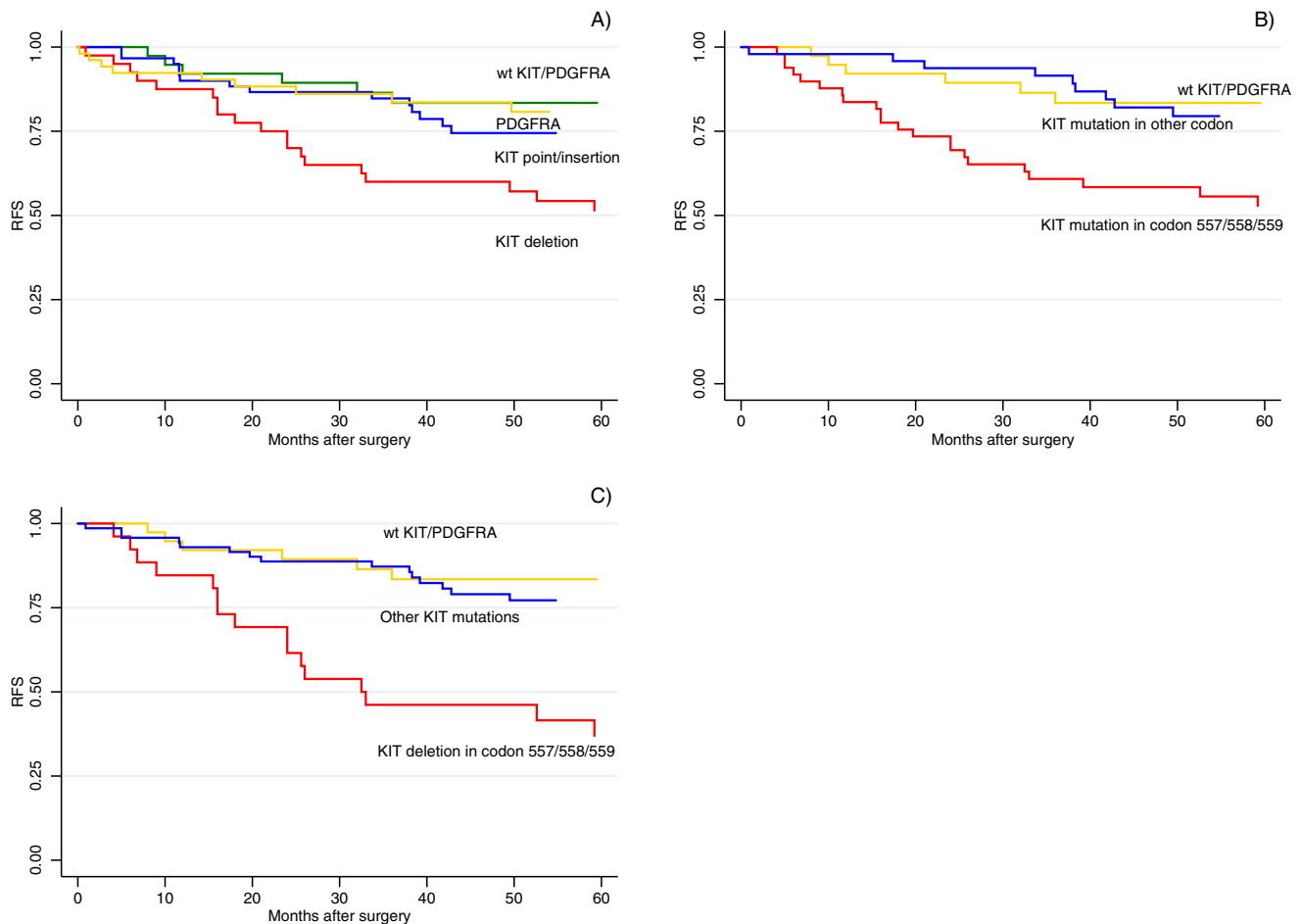


Figure 1. Association between the type of *KIT* mutation, *PDGFRA* and recurrence-free survival (RFS-combined endpoint) after surgery. (A) GISTs with *KIT* mutations, *PDGFRA* mutations or no mutations in either gene; (B) GISTs with *KIT* mutations in exon 11 codons 557 or 558 or 559, in other codons, or no mutations in either gene; (C) GISTs with *KIT* deletions in exon 11 codons 557 or 558 or 559, other *KIT* mutations in exon 11, or *KIT* deletions in exon 11 in codons other than 557, 558 or 559.

risk of recurrence or death (HR [95% CI] = 2.16 [1.14–4.09], $p = 0.017$, HR [95% CI] = 3.98 [2.21–7.19], $p < 0.001$, HR [95% CI] = 2.15 [1.04–4.41], $p = 0.038$, HR [95% CI] = 1.85 [0.99–3.43], $p = 0.053$, HR [95% CI] = 4.11 [2.43–6.95], $p < 0.001$) (Table 3). The treatment variable was statistically significant with a HR [95% CI] of 3.97 [2.08–7.59] ($p < 0.001$). This apparently counterintuitive result was mainly due to the fact that treated patients in this retrospective study were characterized by a worse clinical profile than non-treated patients (*i.e.*, high Miettinen risk score, >5 mitoses, larger tumors).

The presence of *KIT* mutations was associated with a higher risk of recurrence or death (HR [95% CI] = 2.21 [1.03–4.75], $p = 0.042$). In particular, *KIT* deletions were associated with a poorer prognosis than other alterations (HR [95% CI] = 3.65 [1.62–8.22], $p = 0.002$) (Table 3). With regard to the different codons involved, a higher risk of relapse or death was observed in patients with codons 557, 558 or 559 mutations compared to wt

patients or those who had mutations in other sites (HR [95% CI] = 3.68 [1.65–8.24], $p = 0.001$) (Table 3). This difference was even more evident in patients with deletions involving codons 557, 558 or 559 (HR [95% CI] = 5.52 [2.39–12.76], $p < 0.001$) (Table 3).

The results from two multivariable Cox regression models are reported in Table 4. Model 1 considers the prognostic effect of the type of mutations (*KIT* or *PDGFRA*) adjusting for the Miettinen risk score, while Model 2 focuses on the effect of the different types of exon 11 *KIT* mutations on prognosis, once again adjusting for the Miettinen score. After adjusting, *KIT* deletions remained an independent predictor of recurrence or death (HR_{adj} [95% CI] = 2.65 [1.15–6.13], $p = 0.023$) (Table 4 – Model 1), as did *KIT* deletions at codons 557, 558 or 559 (HR_{adj} [95% CI] = 3.29 [1.64–6.64], $p = 0.001$) (Table 4 – Model 2). In the latter model we performed an adjusted comparison between *KIT* deletions in the codons considered and other types of *KIT* mutations or *KIT* deletions in other codons. The risk of recurrence or death for patients

Table 3
Results from univariable analysis using the Cox proportional hazards model.

	HR [95% CI]	p
Gender		
Female	1	
Male	1.45 [0.85–2.47]	0.175
Age at diagnosis, years	1.02 [1.00–1.05]	0.049
Tumor dimension, cm		
≤ 5	1	
6–10	1.21 [0.64–2.25]	0.558
>10	2.16 [1.14–4.09]	0.017
Mitoses		
≤ 5	1	
>5	3.98 [2.21–7.19]	<0.001
Ulceration		
No	1	
Yes	2.15 [1.04–4.41]	0.038
Necrosis		
No	1	
Yes	1.85 [0.99–3.43]	0.053
Atypia		
No	1	
Yes	1.65 [0.85–3.18]	0.135
Miettinen risk score		
Low	1	
High	4.11 [2.43–6.95]	<0.001
Cellularity		
Mixed	2.04 [0.77–5.38]	
Spindle	1.41 [0.58–3.44]	0.149
Epithelioid	1	0.444
Treatment		
No	1	
Adjuvant/neoadjuvant	3.97 [2.08–7.59]	<0.001
Gene mutations		
wt <i>KIT</i> / <i>PDGFRA</i>	1	
<i>KIT</i>	2.21 [1.03–4.75]	0.042
<i>PDGFRA</i>	1.06 [0.42–2.69]	0.904
Type of mutation [<i>KIT</i>/<i>PDGFRA</i>]		
wt <i>KIT</i> / <i>PDGFRA</i>	1	
<i>KIT</i> point/insertion	1.43 [0.61–3.34]	0.412
Deletion	3.65 [1.62–8.22]	0.002
<i>PDGFRA</i>	1.06 [0.42–2.70]	0.8974
Exon 11 <i>KIT</i> mutations		
wt <i>KIT</i> / <i>PDGFRA</i>	1	
In codons 557/558/559	3.68 [1.65–8.24]	0.001
Not in codons 557/558/559	1.12 [0.45–2.79]	0.811
Exon 11 <i>KIT</i> mutations		
wt <i>KIT</i> / <i>PDGFRA</i>	1	
<i>KIT</i> deletion in codons 557/558/559	5.52 [2.39–12.76]	<0.001
<i>KIT</i> point/insertion or deletion	1.33 [0.58–3.07]	0.504
not in codons 557/558/559		

wt – wild type.

with a *KIT* deletion in codons 557, 558 or 559 was more than 3-fold higher than that of patients with other types of mutations or mutations in other positions (HR_{adj} [95% CI] = 3.17 [1.63–6.14], $p = 0.001$) (results not shown).

Survival analysis revealed that the type of mutation only influenced the non treated patient group (Table S4). In univariable analysis, older age, higher number of mitoses, high Miettinen score, and the presence of ulceration were significantly associated with an increased risk of relapse or death

in this group (results not shown). With regard to the molecular variables, a *KIT* deletion was associated with an almost 4-fold increased risk of relapse or death compared to wt patients (HR [95% CI] = 3.71 [1.19–11.62, $p = 0.024$]). Considering *KIT* deletions in codons 557, 558 or 559, the risk was almost 6-fold higher than that of wt patients (HR [95% CI] = 5.81 [1.77–19.06, $p = 0.004$]). After adjusting for the Miettinen score, the risk for *KIT*-deleted patients was 3-fold that of wt cases, but not significant (HR_{adj} [95% CI] = 3.00 [0.92–9.75, $p = 0.068$]) (Table S4, Model 1). *KIT* deletions in codons 557, 558 or 559 were significantly associated with a poorer prognosis with respect to wt tumors (HR_{adj} [95% CI] = 3.94 [1.12–13.81, $p = 0.032$]) (Table S4, Model 2). In treated patients, the only variable associated with RFS was the Miettinen score and none of the molecular variables proved significant even after adjusting for the score (Table S5, Models 1 and 2).

Discussion

The prediction of clinical behavior and the prognosis of patients with GISTs depend largely on classic clinical–pathological risk criteria based on tumor size, mitotic activity and localization of the primary lesion. The mutational status of *KIT* and *PDGFRA* can predict the response to treatment with tyrosine kinase inhibitors,^{16,17} but its role as a prognostic factor remains unclear. Moreover, a significant association has been found between clinical–pathological parameters and molecular alterations, which could also prove to be a prognostic factor.

In our retrospective case series of gastric GISTs, 53.9% and 25.3% of patients showed *KIT* and *PDGFRA* mutations, respectively, while 20.8% had *KIT* and *PDGFRA* wt tumors. These frequencies are lower for *KIT* and higher for *PDGFRA* than the same mutations observed in non gastric GISTs,^{4,5,7,10,21,28} in agreement with results from other studies. Miettinen et al.¹⁴ observed a 22.6% incidence of *PDGFRA* mutations in a series of gastric GISTs, while Wasag et al.²⁹ reported that all *PDGFRA* mutations (25%) found in their GIST case series were observed in gastric GISTs. In Wardelmann et al.'s study,³⁰ all the GISTs with *PDGFRA* mutations (23%) were located in the stomach, whereas tumors with *KIT* mutations (47%) or wt status were also found in the small bowel.

It has been shown that *PDGFRA* mutations in GISTs are associated with a better prognosis.^{7,15,18,21,31} The higher *PDGFRA* mutation frequency observed in gastric GISTs is in accordance with the better prognosis observed for this type of tumor.^{12,30,32} Our study revealed the potential prognostic relevance of the type and position of *KIT* mutations in untreated resected gastric GISTs, in addition to classic pathological criteria such as dimension, mitosis, Miettinen risk stratification, ulceration and necrosis. Tumors with mutations (especially deletions), affecting codons 557, 558 or 559 of *KIT* exon 11 would appear to be a distinct subset of gastric GISTs with malignant clinical

Table 4

Results from two multivariable Cox regression models.

	Model 1		Model 2	
	HR _{adj} [95% CI]	p	HR _{adj} [95% CI]	p
Type of mutation [KIT/PDGFRA]				
wt <i>KIT</i> / <i>PDGFRA</i>	1			
<i>KIT</i> point/insertion	0.99 [0.41–2.39]	0.977		
<i>KIT</i> deletion	2.65 [1.15–6.13]	0.023		
<i>PDGFRA</i>	1.06 [0.42–2.69]	0.906		
Miettinen risk score				
Low	1		1	
High	3.36 [1.90–5.93]	<0.001	3.12 [1.75–5.55]	<0.001
Exon 11 <i>KIT</i> mutations				
wt <i>KIT</i>			1	
<i>KIT</i> deletion in codons 557/558/559			3.29 [1.64–6.64]	0.001
<i>KIT</i> point mutation/insertion or deletion not in codons 557/558/559			1.04 [0.53–2.03]	0.909

wt – wild type.

HR_{adj} – adjusted hazard ratio.

behavior. Deletions involving codons 557, 558 or 559 were associated with a higher risk of relapse or death than that of deletions with no involvement of any of these codons. Moreover, survival analysis showed that patients with these mutations had a lower RFS than those with mutations in other sites. Multiple regression analysis confirmed the independent prognostic value of this finding: adjusting for the clinical covariate of the Miettinen risk stratification system, deletions of exon 11 codons 557, 558 or 559 remained an independent predictor of risk of recurrence or death. The negative prognostic value of exon 11 mutations was significant in untreated patients but not in the smaller group receiving adjuvant or neo-adjuvant treatment. This may be attributable to the high responsiveness of *KIT* exon 11-mutated patients to imatinib.¹⁵

Previous studies have reported similar results for GISTs of different sites, demonstrating that tumors with *KIT* exon 11 deletions are clinically more aggressive than those with exon 11 point mutations. In particular, *KIT* deletions affecting codons 557 or 558 have been associated with metastatic behavior, a high risk of relapse and shorter RFS and OS with respect to other *KIT* exon 11 mutations or deletions involving other codons.^{14,19,21–25,27,31,33}

The higher risk of recurrence or death in patients with GISTs showing abnormalities in exon 11 codons 557 or 558 would seem to be due to the important role of the amino acids encoded by these two codons in *KIT* protein function. A number of juxtamembrane residues are needed to inhibit spontaneous *KIT* phosphorylation. In fact, an increase in phosphorylation could be due to the removal of the side chain of codons 553, 557, 559 or 560. In particular, codon 557 encodes for a juxtamembrane residue responsible for preventing spontaneous receptor phosphorylation and activation. Trp-557-Ala leads to a substantial increase in receptor phosphorylation. Similarly, Lys-558-Pro causes spontaneous receptor phosphorylation which may be higher than stem cell factor-induced wild-type receptor phosphorylation. These mutation-induced conformational receptor

changes lose their inhibitory control of the kinase activity of the ligand-unoccupied *KIT* receptor. Furthermore, codons 557 and/or 558 deletions may lead to a perturbation of kinase autoinhibition via the disruption of protein conformation.^{23,34}

A recent study identified three molecular risk subgroups on the basis of different *KIT*, *PDGFRA* and *BRAF* mutations at different exons.²⁷ We were not able to verify this biological classification because our case series was smaller and also because some alterations were either detected in only a few patients or not at all. We did not find mutations in *BRAF* gene, in agreement with some studies.^{28,35} Conversely, a number of studies reported a 4–13% frequency of *BRAF* mutation (V600E) in *KIT* and *PDGFRA* wt GIST patients,^{8,9,27} the majority of which were of small bowel origin, suggesting a strong predisposition for tumors arising in this part of the gastrointestinal tract.^{8,9} This may explain the absence of *BRAF* mutations in our case series composed entirely of gastric GISTs.

As far as we know, this is the largest study conducted to date on gastric GISTs characterized at molecular level. Its main weakness stems from its retrospective nature, with the obvious difficulty in collecting clinical information and the consequent limitation in the number of patients for whom the analysis of prognosis was possible. Moreover, despite the known disadvantages of a composite endpoint, we decided to use it in our study because of the potential underreporting of disease recurrences related to the non homogeneous availability of follow up in the different centers involved in the study. Finally, findings from the analysis of the treated group should be interpreted with caution due to the consequently smaller sample size on which estimated hazard ratios were based.

In conclusion, we found that *KIT* mutations, especially deletions involving exon 11 codons 557, 558 or 559, in gastric GISTs were associated with a more aggressive phenotype and a higher risk of recurrence or death. This finding underlines the need for a more specific and broader

molecular analysis for GIST patients to identify those at higher risk of relapse who could benefit from adjuvant treatment with imatinib. Furthermore, our data could be used to improve the present classification system by teaming up specific molecular alterations with currently used pathological criteria.

Conflict of interest statement

Riccardo Ricci has received speaker honoraria from Novartis. The remaining authors have no conflicts of interest to declare.

Ethical statement

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. All cases were recruited from 8 member centers of the Italian Gastric Cancer Research Group (GIRCG). Clinical pathological data were supplemented by a review of all available medical and histopathological records archived in GIRCG centers. The study was approved by the Local Ethics Committee of each center.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejso.2016.05.022>.

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